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(57) Abstract

Vaccine compositions comprising a protein that has at least one epitope of human ST receptor protein or a nucleic acid molecule that encodes such a protein are disclosed. Haptenized proteins that comprise at least one epitope of human ST receptor protein and vaccine compositions comprising such protein are disclosed. Killed or inactivated cells or particles that comprise the human ST receptor protein including haptenized killed or inactivated cells or particles that comprise the human ST receptor protein and vaccines made from such compositions are disclosed. Methods of treating individuals who have metastasized colorectal cancer as well as prophylactic methods for treating individuals identified as being susceptible to metastasized colorectal cancer are disclosed.

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METASTATIC COLORECTAL CANCER VACCINE

FIELD OF THE INVENTION

The invention relates to prophylactic and therapeutic vaccines for protecting individuals against metastatic colorectal cancer and for treating individuals who are suffering from metastatic colorectal cancer.

BACKGROUND OF THE INVENTION

Colorectal cancer is the third most common neoplasm worldwide. The mortality rate of newly diagnosed large bowel cancer approaches 50% and there has been little improvement over the past 40 years. Most of this mortality reflects local, regional and distant metastases.

Surgery is the mainstay of treatment for colorectal cancer but recurrence is frequent. Colorectal cancer has proven resistant to chemotherapy, although limited success has been achieved using a combination of 5-fluorouracil and levamisole. Surgery has had the largest impact on survival and, in some patients with limited disease, achieves a cure. However, surgery removes bulk tumor, leaving behind microscopic residual disease which ultimately results in recrudescence.

Early detection of primary, metastatic, and recurrent disease can significantly impact the prognosis of individuals suffering from colorectal cancer. Large bowel cancer diagnosed at an early stage has a significantly better outcome than that diagnosed at more advanced stages. Similarly, diagnosis of

metastatic or recurrent disease earlier potentially carries with it a better prognosis.

Recent discoveries have shown that mutations of the human APC (Adenomatous Polyposis Coli) gene are responsible for both sporadic and familial colorectal cancers. Germ-line mutations of APC are found in inherited familial cancers such as Gardner's syndrome, attenuated adenomatous polyposis coli, heredity flat adenoma syndrome and familial adenomatous polyposis (FAP). FAP is an autosomal dominant inherited disease predisposing the patient to colon cancer. Patients inheriting a single mutant allele of APC develop hundreds to thousands of adenomatous polyps in the second to third decades of life, which if left untreated progress to malignant carcinomas. Genetic linkage analysis localized the APC gene to human chromosome 5q21-q22, a region frequently associated with allelic loss of the wildtype 5q allele. Mutations in APC are also implicated in sporadic colorectal cancers and in extracolonic tumors, such as gastric and small intestinal polyps, osteomas, sarcomas and desmoidal tumors.

There is a need for improved methods of treating individuals suffering from metastasized colon cancer. There is a need for compositions useful to treat individuals suffering from metastasized colon cancer. There is a need for improved methods of preventing a recurrence of metastasized colon cancer in individuals who have been treated for metastasized colon cancer. There is a need for compositions useful to prevent a recurrence of metastasized colon cancer in individuals who have been treated for metastasized colon cancer. There is a need for improved methods of preventing metastasized colon cancer in individuals, particularly those who have been identified as having a genetic predisposition for colon cancer. There is a need for compositions useful for preventing metastasized colon cancer in individuals.

SUMMARY OF THE INVENTION

The invention relates to an isolated protein comprising at least one epitope of human ST receptor protein.

receptor protein. In some embodiments, the nucleic acid molecule encodes a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that comprises the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes human ST receptor protein. In some embodiments, the nucleic acid molecule is a plasmid.

The invention relates to vaccines which comprise such nucleic acid molecules and a pharmaceutically acceptable carrier or diluent.

The invention relates to vectors that comprise nucleic acid molecules that encode a protein comprising at least one epitope of human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein that protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that

In some embodiments, the epitope is an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the human ST receptor protein. In some embodiments, the isolated protein consists of the human ST receptor protein.

The invention relates to vaccines which comprise such proteins and a pharmaceutically acceptable carrier or diluent.

The invention relates to a haptenized protein comprising at least one epitope of human ST receptor protein. In some embodiments, the epitope is an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the human ST receptor protein. In some embodiments, the haptenized protein consists of the human ST receptor protein.

The invention relates to vaccines which comprise such haptenized proteins and a pharmaceutically acceptable carrier or diluent.

The invention relates to nucleic acid molecules that encode a protein comprising at least one epitope of human ST

encodes a protein that comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein that comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein that comprises the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes human ST receptor protein. In some embodiments, the vector is a virus or a bacterial cell. In some embodiments, the vector is a recombinant vaccinia virus.

The invention relates to vaccines which comprise such vectors and a pharmaceutically acceptable carrier or diluent.

The invention relates to killed or inactivated cells or particles that comprise a protein comprising at least one epitope of human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise the extracellular domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles vector comprise the transmembrane domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise the cytoplasmic domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles vector is a killed or inactivated colorectal tumor cells.

The invention relates to vaccines which comprise such killed or inactivated cells or particles and a pharmaceutically acceptable carrier or diluent.

The invention relates to haptenized killed or inactivated cells or particles that comprise a protein comprising at least one epitope of human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise the extracellular domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles vector comprise the transmembrane domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles vector is a killed or inactivated colorectal tumor cells.

The invention relates to vaccines which comprise such haptenized killed or inactivated cells or particles and a pharmaceutically acceptable carrier or diluent.

The present invention relates to methods of treating individuals suffering from metastasized colorectal cancer. The method of the present invention provides administering to such an individual a therapeutically effective amount of a vaccine of the invention. The invention relates to the use of such vaccines as immunotherapeutics.

The present invention relates to methods of treating individuals susceptible metastasized colorectal cancer. The method of the present invention provides administering to such an individual an amount of a vaccine of the invention effective to prevent or combat metastasized colorectal cancer. The present invention relates to the use of the vaccines of the invention prophylactically.

DETAILED DESCRIPTION OF THE INVENTION

U.S. Serial Number 08/141,892 filed on October 26, 1993 (which is scheduled to issue on May 21, 1996 as U.S. Patent Number 5,518,888), U.S. Serial Number 08/305,056 filed on September 13, 1994, and PCT Application Serial Number PCT/US94/12232 filed October 26, 1994, which are each incorporated herein by reference, describe compositions for and methods of treating, imaging and detecting metastasized colon cancer.

As used herein, the terms "ST receptor" and "guanylin cyclase C" are interchangeable and meant to refer to the receptors found on colorectal cells, including local and metastasized colorectal cancer cells, which bind to ST. In normal individuals, ST receptors are found exclusively in cells of intestine, in particular in cells in the duodenum, small intestine (jejunum and ileum), the large intestine, colon (cecum, ascending colon, transverse colon, descending colon and sigmoid colon) and rectum.

As used herein, the term "colorectal cancer" is meant to include the well-accepted medical definition that defines colorectal cancer as a medical condition characterized by cancer of cells of the intestinal tract below the small intestine (i.e. the large intestine (colon), including the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon, and rectum). Additionally, as used herein, the term "colorectal cancer" is meant to further include medical conditions which are characterized by cancer of cells of the duodenum and small intestine (jejunum and ileum). The definition of colorectal cancer used herein is more expansive

than the common medical definition but is provided as such since the cells of the duodenum and small intestine also contain ST receptors and are therefore amenable to the methods of the present invention using the compounds of the present invention.

As used herein, the term "metastasis" is meant to refer to the process in which cancer cells originating in one organ or part of the body relocate to another part of the body and continue to replicate. Metastasized cells subsequently form tumors which may further metastasize. Metastasis thus refers to the spread of cancer from the part of the body where it originally occurs to other parts of the body. The present invention relates to methods of delivering active agents to metastasized colorectal cancer cells.

As used herein, the term "metastasized colorectal cancer cells" is meant to refer to colorectal cancer cells which have metastasized; colorectal cancer cells localized in a part of the body other than the duodenum, small intestine (jejunum and ileum), large intestine (colon), including the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon, and rectum.

As used herein, "an individual is suspected of being susceptible to metastasized colorectal cancer" is meant to refer to an individual who is at an above-average risk of developing metastasized colorectal cancer. Examples of individuals at a particular risk of developing metastasized colorectal cancer are those whose family medical history indicates above average incidence of colorectal cancer among family members and/or those who have already developed colorectal cancer and have been effectively treated who therefore face a risk of relapse and recurrence. Other factors which may contribute to an above-average risk of developing metastasized colorectal cancer which would thereby lead to the classification of an individual as being suspected of being susceptible to metastasized colorectal cancer may be based upon an individual's specific genetic, medical and/or behavioral background and characteristics.

Heat stable toxin ST, which is produced by *E. coli*, as well as other organisms, is responsible for endemic diarrhea in developing countries and travelers diarrhea. ST induces intestinal secretion by binding to specific receptors, ST receptors, in the apical brush border membranes of the mucosal cells lining the intestinal tract. Binding of ST to ST receptors is non-covalent and occurs in a concentration-dependent and saturable fashion. Once bound, ST-ST receptor complexes appear to be internalized by intestinal cells, i.e. transported from the surface into the interior of the cell. Binding of ST to ST receptors triggers a cascade of biochemical reactions in the apical membrane of these cells resulting in the production of a signal which induces intestinal cells to secrete fluids and electrolytes, resulting in diarrhea.

ST receptors are unique in that they are only localized in the apical brush border membranes of the cells lining the intestinal tract. Indeed, they are not found in any other cell type in placental mammals. In addition, ST receptors are almost exclusively localized to the apical membranes, with little being found in the basolateral membranes on the sides of intestinal cells.

Mucosal cells lining the intestine are joined together by tight junctions which form a barrier against the passage of intestinal contents into the blood stream and components of the blood stream into the intestinal lumen. Therefore, the apical location of ST receptors isolates these receptors from the circulatory system so that they may be considered to exist separate from the rest of the body; essentially the "outside" of the body. Therefore, the rest of the body is considered "outside" the intestinal tract, i.e. extraintestinal. Compositions administered "outside" the intestinal tract are maintained apart and segregated from the only cells which normally express ST receptors. Conversely, tissue samples taken from tissue outside of the intestinal tract, i.e. extraintestinal tissue samples, do not normally contain cells which express ST receptors.

In individuals suffering from colorectal cancer, the cancer cells are often derived from cells that produce and display the ST receptor and these cancer cells continue to produce and display the ST receptor on their cell surfaces. Indeed, T84 cells, which are human colonic adenocarcinoma cells isolated from lung metastases, express ST receptors on their cell surface. Similarly, HT29glu-cells, which are human colonic adenocarcinoma cells, express receptors for ST. Thus, in individuals suffering from colorectal cancer, some metastasized intestinal cancer cells express ST receptors.

An effort was undertaken to determine the proportion of colorectal tumors which have the ST receptor. Each of the tumors tested were independently confirmed to be colorectal cancer by standard techniques of surgical pathology. Every one of the colorectal cancer tumors tested, including local colorectal tumors and metastasized colorectal tumors (liver, lung, lymph node, peritoneum, ovary) possessed ST receptors. In each case, the affinity and density of receptors was amenable for targeting. Normal liver, lymph node, peritoneum, gall bladder, ovary, stomach, kidney and lung cells were found not to possess ST receptors.

When such cancer cells metastasize, the metastasized cancer cells continue to produce and display the ST receptor. The expression of ST receptors on the surfaces of metastatic tumors provides a target which can be used to distinguish the metastasized colorectal cancer cells from normal extraintestinal cells. This target is useful in the detection, imaging and treatment of metastasized colorectal cancer.

According to the present invention, the ST receptor protein serves as a target against which a protective and therapeutic immune response can be induced. Specifically, vaccines are provided which induce an immune response against the ST receptor protein. The vaccines of the invention include, but are not limited to, the following vaccine technologies:

1) DNA vaccines, i.e. vaccines in which DNA that encodes at least an epitope from ST receptor protein is

administered to an individual's cells where the epitope is expressed and serves as a target for an immune response;

2) infectious vector mediated vaccines such as recombinant adenovirus, vaccinia, *Salmonella*, and BCG wherein the vector carries genetic information that encodes at least an epitope of ST receptor protein such that when the infectious vector is administered to an individual, the epitope is expressed and serves as a target for an immune response;

3) killed or inactivated vaccines which a) comprise either killed cells or inactivated viral particles that display at least an epitope of the ST receptor protein and b) when administered to an individual serves as a target for an immune response;

3) haptenized killed or inactivated vaccines which a) comprise either killed cells or inactivated viral particles that display at least an epitope of the ST receptor, b) are haptenized to be more immunogenic and c) when administered to an individual serves as a target for an immune response;

4) subunit vaccines which are vaccines that include protein molecules that include at least an epitope the ST receptor protein; and

5) haptenized subunit vaccines which are vaccines that a) include protein molecules that include at least an epitope the ST receptor protein and b) are haptenized to be more immunogenic.

The present invention relates to administering to an individual a protein or nucleic acid molecule that comprises or encodes, respectively, an immunogenic epitope against which an therapeutic and prophylactic immune response can be induced. Such epitopes are generally at least 6-8 amino acids in length. The vaccines of the invention therefore comprise proteins which are at least, or nucleic acids which encode at least, 6-8 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, the entire ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least 10 to about

1000 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, about 25 to about 500 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, about 50 to about 400 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, about 100 to about 300 amino acids in length from ST receptor protein. In preferred embodiments, fragments of ST receptor protein that include the extracellular domain are provided.

The present invention relates to compositions for and methods of treating individuals who are known to have metastasized colorectal cancer. Metastasized colorectal cancer may be diagnosed by those having ordinary skill in the art using art accepted clinical and laboratory pathology protocols and/or those described in U.S. Serial Number 08/141,892 filed on October 26, 1993, U.S. Serial Number 08/305,056 filed on September 13, 1994, and PCT Application Serial Number PCT/US94/12232 filed October 26, 1994. The present invention provides an immunotherapeutic vaccine useful to treat individuals who have been diagnosed as suffering from metastasized colorectal cancer. The immunotherapeutic vaccines of the present invention may be administered in combination with other therapies including, but not limited to those described in U.S. Serial Number 08/141,892 filed on October 26, 1993, U.S. Serial Number 08/305,056 filed on September 13, 1994, and PCT Application Serial Number PCT/US94/12232 filed October 26, 1994.

The present invention relates to compositions for and methods of preventing metastatic colorectal cancer in individual is suspected of being susceptible to metastasized colorectal cancer. Such individuals include those whose family medical history indicates above average incidence of colorectal cancer among family members and/or those who have already developed colorectal cancer and have been effectively

treated who therefore face a risk of relapse and recurrence. Such individuals include those which have been diagnosed as having colorectal cancer including localized only or localized and metastasized colorectal cancer which has been resected or otherwise treated. Such individuals also include those with an elevated risk as ascertained by genetic evaluation. For example, individuals with APC mutations can be identified following the U.S. Patent Number 5,352,775 issued October 4, 1992 to Albertsen et al., which is incorporated herein by reference. Furthermore, such individuals include: those suffering from inflammatory bowel disease, particularly those with ulcerative colitis; those with colonic polyps; those with familial adenomatous polyposis, a heritable mutation predisposing patients to develop large numbers of intestinal polyps; those with Peutz-Jeghers syndrome; those with hereditary nonpolyposis coli, a heritable mutation which predisposes people to develop colon carcinoma; those with Turcot syndrome-colon carcinoma in conjunction with independent tumors of the central nervous system; and individuals engaging in rectal intercourse. The vaccines of the present invention may be to susceptible individuals prophylactically to prevent and combat colorectal cancer metastasis.

The invention relates to compositions which are the active components of such vaccines or required to make the active components, to methods of making such compositions including the active components, and to methods of making and using vaccines.

The nucleotide sequence that encodes human ST receptor protein is disclosed as SEQ ID NO:1. The amino acid sequence of human ST receptor is also disclosed in SEQ ID NO:1. Generally, the extracellular domain refers to the amino acids about 24 to about 454. The transmembrane region refers to amino acids about 455 to about 475. The cytoplasmic domain refers to amino acids about 476 to about 1093.

Accordingly, some aspects of the invention relate to isolated proteins that comprise at least one ST receptor epitope. The epitope may be from the ST receptor extracellular

domain, transmembrane domain or cytoplasmic domain. In preferred embodiments, the protein comprises at least one epitope from the extracellular domain. The protein may comprise ST receptor protein sequences or consist of ST receptor protein sequences. The protein may comprise the entire ST receptor protein, consist of the entire ST receptor protein, comprise a fragment of the ST receptor protein, or consist of a fragment of the ST receptor protein. In some preferred embodiments, the protein is a soluble form of the extracellular domain. In some preferred embodiments, the protein is a soluble form of the extracellular domain with a portion of the transmembrane domain.

Some aspects of the invention relate to the above described isolated proteins which are haptenized to render them more immunogenic. That is, some aspects of the invention relate to haptenized proteins that comprise at least one ST receptor epitope. The epitope may be from the ST receptor extracellular domain, transmembrane domain or cytoplasmic domain. The protein may comprise ST receptor protein sequences or consist of ST receptor protein sequences. The protein may comprise the entire ST receptor protein, consist of the entire ST receptor protein, comprise a fragment of the ST receptor protein, or consist of a fragment of the ST receptor protein. In some preferred embodiments, the haptenized protein comprises a soluble form of the extracellular domain. In some preferred embodiments, the haptenized protein is a soluble form of the extracellular domain with a portion of the transmembrane domain.

Some aspects of the invention nucleic acid molecules that encode the above described isolated proteins.

Accordingly, some aspects of the invention relate to isolated nucleic acid molecules that encode proteins that comprise at least one ST receptor epitope. The epitope may be from the ST receptor extracellular domain, transmembrane domain or cytoplasmic domain. In preferred embodiments, the isolated nucleic acid molecules encodes a protein that comprises at least one epitope from the extracellular domain. The isolated

nucleic acid molecule may encode a protein that comprises or consists of ST receptor protein sequences. The isolated nucleic acid molecule may encode a protein that comprises or consists of the entire ST receptor protein, or a protein that comprises or consists of a fragment of the ST receptor protein. In some embodiments, the isolated nucleic acid molecule encodes non-ST receptor protein sequences which are useful to render the ST receptor protein sequences more immunogenic.

Naked DNA vaccines are described in PCT/US90/01515, which is incorporated herein by reference. Others teach the use of liposome mediated DNA transfer, DNA delivery using microprojectiles (U.S. Patent No. 4,945,050 issued July 31, 1990 to Sanford et al., which is incorporated herein by reference), and DNA delivery using electroporation. In each case, the DNA may be plasmid DNA that is produced in bacteria, isolated and administered to the animal to be treated. The plasmid DNA molecules are taken up by the cells of the animal where the sequences that encode the protein of interest are expressed. The protein thus produced provides a therapeutic or prophylactic effect on the animal.

The use of vectors including viral vectors and other means of delivering nucleic acid molecules to cells of an individual in order to produce a therapeutic and/or prophylactic immunological effect on the individual are similarly well known. Recombinant vaccines that employ vaccinia vectors are, for example, disclosed in U.S. Patent Number 5,017,487 issued May 21, 1991 to Stunnenberg *et al.* which is incorporated herein by reference.

In some cases, tumor cells from the patient are killed or inactivated and administered as a vaccine product. Berd *et al.* May 1986 *Cancer Research* **46**:2572-2577 and Berd *et al.* May 1991 *Cancer Research* **51**:2731-2734, which are incorporated herein by reference, describes the preparation and use of tumor cell based vaccine products. According to some aspects of the present invention, the methods and techniques described in Berd *et al.* are adapted by using colorectal cancer cells instead of melanoma cells.

The manufacture and use of subunit vaccines are well known. One having ordinary skill in the art can isolate the nucleic acid molecule that encode ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. Once isolated, the nucleic acid molecule can be inserted it into an expression vector using standard techniques and readily available starting materials. Rudner *et al.* May 1995 *Proc. Natl. Acad. Sci. USA* 92:5169-5173 disclosed the cloning and expression of the extracellular domain of human ST receptor and purification of the same using a Flag immunoaffinity epitope and antibody therefor.

The recombinant expression vector that comprises a nucleotide sequence that encodes the nucleic acid molecule that encode ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. As used herein, the term "recombinant expression vector" is meant to refer to a plasmid, phage, viral particle or other vector which, when introduced into an appropriate host, contains the necessary genetic elements to direct expression of the coding sequence that encodes the protein. The coding sequence is operably linked to the necessary regulatory sequences. Expression vectors are well known and readily available. Examples of expression vectors include plasmids, phages, viral vectors and other nucleic acid molecules or nucleic acid molecule containing vehicles useful to transform host cells and facilitate expression of coding sequences. The recombinant expression vectors of the invention are useful for transforming hosts to prepare recombinant expression systems for preparing the isolated proteins of the invention.

The present invention relates to a host cell that comprises the recombinant expression vector that includes a nucleotide sequence that encodes the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. Host cells for use in well known recombinant expression systems for production of proteins are well known and readily available. Examples of host cells include bacteria cells such as *E. coli*, yeast cells such as *S.*

cerevisiae, insect cells such as *S. frugiperda*, non-human mammalian tissue culture cells chinese hamster ovary (CHO) cells and human tissue culture cells such as HeLa cells.

The present invention relates to a transgenic non-human mammal that comprises the recombinant expression vector that comprises a nucleic acid sequence that encodes the proteins of the invention. Transgenic non-human mammals useful to produce recombinant proteins are well known as are the expression vectors necessary and the techniques for generating transgenic animals. Generally, the transgenic animal comprises a recombinant expression vector in which the nucleotide sequence that encodes the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof operably linked to a mammary cell specific promoter whereby the coding sequence is only expressed in mammary cells and the recombinant protein so expressed is recovered from the animal's milk.

In some embodiments, for example, one having ordinary skill in the art can, using well known techniques, insert such DNA molecules into a commercially available expression vector for use in well known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, CA) may be used for production of collagen in *E. coli*. The commercially available plasmid pYES2 (Invitrogen, San Diego, CA) may, for example, be used for production in *S. cerevisiae* strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, CA) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA I (Invitrogen, San Diego, CA) may, for example, be used for production in mammalian cells such as Chinese Hamster Ovary cells. One having ordinary skill in the art can use these commercial expression vectors and systems or others to produce the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof using routine techniques and readily available starting materials. (*See e.g.*, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold

Spring Harbor Press (1989) which is incorporated herein by reference.) Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

One having ordinary skill in the art may use other commercially available expression vectors and systems or produce vectors using well known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers, are readily available and known in the art for a variety of hosts. *See e.g.*, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989).

A wide variety of eukaryotic hosts are also now available for production of recombinant foreign proteins. As in bacteria, eukaryotic hosts may be transformed with expression systems which produce the desired protein directly, but more commonly signal sequences are provided to effect the secretion of the protein. Eukaryotic systems have the additional advantage that they are able to process introns which may occur in the genomic sequences encoding proteins of higher organisms. Eukaryotic systems also provide a variety of processing mechanisms which result in, for example, glycosylation, carboxy-terminal amidation, oxidation or derivatization of certain amino acid residues, conformational control, and so forth.

Commonly used eukaryotic systems include, but is not limited to, yeast, fungal cells, insect cells, mammalian cells, avian cells, and cells of higher plants. Suitable promoters are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, *e.g.* the baculovirus polyhedron promoter. As above, promoters can be either constitutive or inducible. For example, in mammalian systems, the mouse metallothionein promoter can be induced by the addition of heavy metal ions.

The particulars for the construction of expression systems suitable for desired hosts are known to those in the

art. Briefly, for recombinant production of the protein, the DNA encoding the polypeptide is suitably ligated into the expression vector of choice. The DNA is operably linked to all regulatory elements which are necessary for expression of the DNA in the selected host. One having ordinary skill in the art can, using well known techniques, prepare expression vectors for recombinant production of the polypeptide.

The expression vector including the DNA that encodes the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof is used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign DNA takes place. The protein of the present invention thus produced is recovered from the culture, either by lysing the cells or from the culture medium as appropriate and known to those in the art. One having ordinary skill in the art can, using well known techniques, the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof that is produced using such expression systems. The methods of purifying the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof using antibodies which specifically bind to the protein are well known. Antibodies which specifically bind to a particular protein may be used to purify the protein from natural sources using well known techniques and readily available starting materials. Such antibodies may also be used to purify the protein from material present when producing the protein by recombinant DNA methodology. The present invention relates to antibodies that bind to an epitope which is present on the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. As used herein, the term "antibody" is meant to refer to complete, intact antibodies, and Fab fragments and $F(ab)_2$ fragments thereof. Complete, intact antibodies include monoclonal antibodies such as murine monoclonal antibodies, chimeric antibodies and humanized antibodies. Antibodies that bind to an epitope which

is present on the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof are useful to isolate and purify the protein from both natural sources or recombinant expression systems using well known techniques such as affinity chromatography. Immunoaffinity techniques generally are described in Waldman *et al.* 1991 *Methods of Enzymol.* 195:391-396, which is incorporated herein by reference. Antibodies are useful to detect the presence of such protein in a sample and to determine if cells are expressing the protein. The production of antibodies and the protein structures of complete, intact antibodies, Fab fragments and $F(ab)_2$ fragments and the organization of the genetic sequences that encode such molecules are well known and are described, for example, in Harlow, E. and D. Lane (1988) *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. which is incorporated herein by reference. Briefly, for example, the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, or an immunogenic fragment thereof is injected into mice. The spleen of the mouse is removed, the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, or hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, the hybridoma which produces them is cultured to produce a continuous supply of antibodies.

In some embodiments of the invention, transgenic non-human animals are generated. The transgenic animals according to the invention contain nculeotides that encode the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof under the regulatory control of a mammary specific promoter. One having ordinary skill in the art using standard techniques, such as those taught in U.S. Patent No. 4,873,191 issued October 10, 1989 to Wagner and U.S. Patent No. 4,736,866 issued

April 12, 1988 to Leder, both of which are incorporated herein by reference, can produce transgenic animals which produce the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. Preferred animals are goats and rodents, particularly rats and mice.

In addition to producing these proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof of the invention. Such techniques are well known to those having ordinary skill in the art and are useful if derivatives which have substitutions not provided for in DNA-encoded protein production.

In some embodiments, the protein that makes up a subunit vaccine or the cells or particles of a killed or inactivated vaccine may be haptenized to increase immunogenicity. In some cases, the haptenization is the conjugation of a larger molecular structure to the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. In some cases, tumor cells from the patient are killed and haptenized as a means to make an effective vaccine product. In cases in which other cells, such as bacteria or eukaryotic cells which are provided with the genetic information to make and display the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, are killed and used as the active vaccine component, such cells are haptenized to increase immunogenicity. Haptenization is well known and can be readily performed.

Methods of haptenizing cells generally and tumor cells in particular are described in Berd *et al.* May 1986 *Cancer Research* 46:2572-2577 and Berd *et al.* May 1991 *Cancer Research* 51:2731-2734, which are incorporated herein by reference. Additional haptenization protocols are disclosed in Miller *et al.* 1976 *J. Immunol.* 117(5:1):1591-1526.

Haptenization compositions and methods which may be adapted to be used to prepare haptenized ST immunogens according to the present invention include those described in the following U.S. Patents which are each incorporated herein by reference: U.S. Patent Number 5,037,645 issued August 6, 1991 to Strahilevitz; U.S. Patent Number 5,112,606 issued May 12, 1992 to Shiosaka *et al.*; U.S. Patent Number 4,526716 issued July 2, 1985 to Stevens; U.S. Patent Number 4,329,281 issued May 11, 1982 to Christenson *et al.*; and U.S. Patent Number 4,022,878 issued May 10, 1977 to Gross. Peptide vaccines and methods of enhancing immunogenicity of peptides which may be adapted to modify ST immunogens of the invention are also described in Francis *et al.* 1989 *Methods of Enzymol.* 178:659-676, which is incorporated herein by reference. Sad *et al.* 1992 *Immunolology* 76:599-603, which is incorporated herein by reference, teaches methods of making immunotherapeutic vaccines by conjugating gonadotropin releasing hormone to diphtheria toxoid. ST immunogens may be similarly conjugated to produce an immunotherapeutic vaccine of the present invention. MacLean *et al.* 1993 *Cancer Immunol. Immunother.* 36:215-222, which is incorporated herein by reference, describes conjugation methodologies for producing immunotherapeutic vaccines which may be adaptable to produce an immunotherapeutic vaccine of the present invention. The hapten is keyhole limpet hemocyanin which may be conjugated to an ST immunogen.

As used herein, the term "ST receptor immunogen" is meant to refer to the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, haptenized ST receptor protein or a haptenized fragment thereof or a haptenized protein that comprises the ST receptor protein or a haptenized fragment thereof, cells and particles which display at least one ST receptor epitope, and haptenized cells and haptenized particles which display at least one ST receptor epitope

Vaccines according to some aspects of the invention comprise a pharmaceutically acceptable carrier in combination with an ST receptor immunogen. Pharmaceutical formulations are

well known and pharmaceutical compositions comprising such proteins may be routinely formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference. The present invention relates to an injectable pharmaceutical composition that comprises a pharmaceutically acceptable carrier and an ST receptor immunogen. The ST receptor immunogen is preferably sterile and combined with a sterile pharmaceutical carrier.

In some embodiments, for example, the ST receptor immunogen can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.

An injectable composition may comprise the ST receptor immunogen in a diluting agent such as, for example, sterile water, electrolytes/dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and polyethylene glycol. The injectable must be sterile and free of pyrogens.

The vaccines of the present invention may be administered by any means that enables the immunogenic agent to be presented to the body's immune system for recognition and induction of an immunogenic response. Pharmaceutical compositions may be administered parenterally, i.e., intravenous, subcutaneous, intramuscular.

Dosage varies depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind

of concurrent treatment, frequency of treatment, and the effect desired. An amount of immunogen is delivered to induce a protective or therapeutically effective immune response. Those having ordinary skill in the art can readily determine the range and optimal dosage by routine methods.

SEQ ID:1

-77
tggagtgggc tgagggactc cactagaggc tgtccatctg gattccctgc ctccctagga
gcccaacaga gcaaagcaag tgggcacaag gagtatggtt ctaacgtgat tggggtc

1/1 31/11
ATG AAG ACG TTG CTG TTG GAC TTG GCT TTG TGG TCA CTG CTC TTC CAG CCC GGG TGG CTG
Met lys thr leu leu leu asp leu ala leu trp ser leu leu phe gln pro gly trp leu

61/21 91/31
TCC TTT AGT TCC CAG GTG AGT CAG AAC TGC CAC AAT GGC AGC TAT GAA ATC AGC GTC CTG
ser phe ser ser gln val ser gln asn cys his asn gly ser tyr glu ile ser val leu

121/41 151/51
ATG ATG GGC AAC TCA GCC TTT GCA GAG CCC CTG AAA AAC TTG GAA GAT GCG GTG AAT GAG
met met gly asn ser ala phe ala glu pro leu lys asn leu glu asp ala val asn glu

181/61 211/71
GGG CTG GAA ATA GTG AGA GGA CGT CTG CAA AAT GCT GGC CTA AAT GTG ACT GTG AAC GCT
gly leu glu ile val arg gly arg leu gln asn ala gly leu asn val thr val asn ala

241/81 271/91
ACT TTC ATG TAT TCG GAT GGT CTG ATT CAT AAC TCA GGC GAC TGC CGG AGT AGC ACC TGT
thr phe met tyr ser asp gly leu ile his asn ser gly asp cys arg ser ser thr cys

301/101 331/111
GAA GGC CTC GAC CTA CTC AGG AAA ATT TCA AAT GCA CAA CGG ATG GGC TGT GTC CTC ATA
glu gly leu asp leu leu arg lys ile ser asn ala gln arg met gly cys val leu ile

361/121 391/131
GGG CCC TCA TGT ACA TAC TCC ACC TTC CAG ATG TAC CTT GAC ACA GAA TTG AGC TAC CCC
gly pro ser cys thr tyr ser thr phe gln met tyr leu asp thr glu leu ser tyr pro

421/141 451/151
ATG ATC TCA GCT GGA AGT TTT GGA TTG TCA TGT GAC TAT AAA GAA ACC TTA ACC AGG CTG
met ile ser ala gly ser phe gly leu ser cys asp tyr lys glu thr leu thr arg leu

481/161 511/171
ATG TCT CCA GCT AGA AAG TTG ATG TAC TTC TTG GTT AAC TTT TGG AAA ACC AAC GAT CTG
met ser pro ala arg lys leu met tyr phe leu val asn phe trp lys thr asn asp leu

541/181 571/191
CCC TTC AAA ACT TAT TCC TGG AGC ACT TCG TAT GTT TAC AAG AAT GGT ACA GAA ACT GAG
pro phe lys thr tyr ser trp ser thr ser tyr val tyr lys asn gly thr glu thr glu

601/201 631/211
GAC TGT TTC TGG TAC CTT AAT GCT CTG GAG GCT AGC GTT TCC TAT TTC TCC CAC GAA CTC
asp cys phe trp tyr leu asn ala leu glu ala ser val ser tyr phe ser his glu leu

661/221 691/231
GGC TTT AAG GTG GTG TTA AGA CAA GAT AAG GAG TTT CAG GAT ATC TTA ATG GAC CAC AAC
gly phe lys val val leu arg gln asp lys glu phe gln asp ile leu met asp his asn

721/241 751/251
AGG AAA AGC AAT GTG ATT ATT ATG TGT GGT GGT CCA GAG TTC CTC TAC AAG CTG AAG GGT
arg lys ser asn val ile ile met cys gly gly pro glu phe leu tyr lys leu lys gly

781/261 811/271
GAC CGA GCA GTG GCT GAA GAC ATT GTC ATT ATT CTA GTG GAT CTT TTC AAT GAC CAG TAC
asp arg ala val ala glu asp ile val ile ile leu val asp leu phe asn asp gln tyr

841/281 871/291
TTG GAG GAC AAT GTC ACA GCC CCT GAC TAT ATG AAA AAT GTC CTT GTT CTG ACG CTG TCT
leu glu asp asn val thr ala pro asp tyr met lys asn val leu val leu thr leu ser

901/301 931/311
CCT GGG AAT TCC CTT CTA AAT AGC TCT TTC TCC AGG AAT CTA TCA CCA ACA AAA CGA GAC

pro gly asn ser leu leu asn ser ser phe ser arg asn leu ser pro thr lys arg asp

961/321 991/331
TTT CGT CTT GCC TAT TTG AAT GGA ATC CTC GTC TTT GGA CAT ATG CTG AAG ATA TTT CTT
phe arg leu ala tyr leu asn gly ile leu val phe gly his met leu lys ile phe leu

1021/341 1051/351
GAA AAT GGA GAA AAT ATT ACC ACC CCC AAA TTT GCT CAT GCC TTC AGG AAT CTC ACT TTT
glu asn gly glu asn ile thr thr pro lys phe ala his ala phe arg asn leu thr phe

1081/361 1111/371
GAA GGG TAT GAC GGT CCA GTG ACC TTG GAT GAC TGG GGG GAT GTT GAC AGT ACC ATG GTG
glu gly tyr asp gly pro val thr leu asp asp trp gly asp val asp ser thr met val

1141/381 1171/391
CTT CTG TAT ACC TCT GTG GAC ACC AAG AAA TAC AAG GTT CTT TTG ACC TAT GAT ACC CAC
leu leu tyr thr ser val asp thr lys lys tyr lys val leu leu thr tyr asp thr his

1201/401 1231/411
GTA AAT AAG ACC TAT CCT GTG GAT ATG AGC CCC ACA TTC ACT TGG AAG AAC TCT AAA CTT
val asn lys thr tyr pro val asp met ser pro thr phe thr trp lys asn ser lys leu

1261/421 1291/431
CCT AAT GAT ATT ACA GGC CGG GGC CCT CAG ATC CTG ATG ATT GCA GTC TTC ACC CTC ACT
pro asn asp ile thr gly arg gly pro gln ile leu met ile ala val phe thr leu thr

1321/441 1351/451
GGA GCT GTG GTG CTG CTC CTG CTC GTC GCT CTC CTG ATG CTC AGA AAA TAT AGA AAA GAT
gly ala val val leu leu leu leu val ala leu leu met leu arg lys tyr arg lys asp

1381/461 1411/471
TAT GAA CTT CGT CAG AAA AAA TGG TCC CAC ATT CCT CCT GAA AAT ATC TTT CCT CTG GAG
tyr glu leu arg gln lys lys trp ser his ile pro pro glu asn ile phe pro leu glu

1441/481 1471/491
ACC AAT GAG ACC AAT CAT GTT AGC CTC AAG ATC GAT GAT GAC AAA AGA CGA GAT ACA ATC
thr asn glu thr asn his val ser leu lys ile asp asp asp lys arg arg asp thr ile

1501/501 1531/511
CAG AGA CTA CGA CAG TGC AAA TAC GTC AAA AAG CGA GTG ATT CTC AAA GAT CTC AAG CAC
gln arg leu arg gln cys lys tyr val lys lys arg val ile leu lys asp leu lys his

1561/521 1591/531
AAT GAT GGT AAT TTC ACT GAA AAA CAG AAG ATA GAA TTG AAC AAG TTG CTT CAG ATT GAC
asn asp gly asn phe thr glu lys gln lys ile glu leu asn lys leu leu gln ile asp

1621/541 1651/551
TAT TAC ACC CTA ACC AAG TTC TAC GGG ACA GTG AAA CTG GAT ACC ATG ATC TTC GGG GTG
tyr tyr thr leu thr lys phe tyr gly thr val lys leu asp thr met ile phe gly val

1681/561 1711/571
ATA GAA TAC TGT GAG AGA GGA TCC CTC CGG GAA GTT TTA AAT GAC ACA ATT TCC TAC CCT
ile glu tyr cys glu arg gly ser leu arg glu val leu asn asp thr ile ser tyr pro

1741/581 1771/591
GAT GGC ACA TTC ATG GAT TGG GAG TTT AAG ATC TCT GTC TTG TAT GAC ATT GCT AAG GGA
asp gly thr phe met asp trp glu phe lys ile ser val leu tyr asp ile ala lys gly

1801/601 1831/611
ATG TCA TAT CTG CAC TCC AGT AAG ACA GAA GTC CAT GGT CGT CTG AAA TCT ACC AAC TGC
met ser tyr leu his ser ser lys thr glu val his gly arg leu lys ser thr asn cys

1861/621 1891/631
GTA GTG GAC AGT AGA ATG GTG GTG AAG ATC ACT GAT TTT GGC TGC AAT TCC ATT TTG CCT
val val asp ser arg met val val lys ile thr asp phe gly cys asn ser ile leu pro

1921/641 1951/651
CCA AAA AAG GAC CTG TGG ACA GCT CCA GAG CAC CTC CGC CAA GCC AAC ATC TCT CAG AAA
pro lys lys asp leu trp thr ala pro glu his leu arg gln ala asn ile ser gln lys

1981/661 2011/671
GGA GAT GTG TAC AGC TAT GGG ATC ATC GCA CAG GAG ATC ATT CTG CGG AAA GAA ACC TTC
gly asp val tyr ser tyr gly ile ile ala gln glu ile ile leu arg lys glu thr phe

2041/681 2071/691
TAC ACT TTG AGC TGT CGG GAC CGG AAT GAG AAG ATT TTC AGA GTG GAA AAT TCC AAT GGA
tyr thr leu ser cys arg asp arg asn glu lys ile phe arg val glu asn ser asn gly

2101/701 2131/711
ATG AAA CCC TTC CGC CCA GAT TTA TTC TTG GAA ACA GCA GAG GAA AAA GAG CTA GAA GTG
met lys pro phe arg pro asp leu phe leu glu thr ala glu glu lys glu leu glu val

2161/721 2191/731
TAC CTA CTT GTA AAA AAC TGT TGG GAG GAA GAT CCA GAA AAG AGA CCA GAT TTC AAA AAA
tyr leu leu val lys asn cys trp glu glu asp pro glu lys arg pro asp phe lys lys

2221/741 2251/751
ATT GAG ACT ACA CTT GCC AAG ATA TTT GGA CTT TTT CAT GAC CAA AAA AAT GAA AGC TAT
ile glu thr thr leu ala lys ile phe gly leu phe his asp gln lys asn glu ser tyr

2281/761 2311/771
ATG GAT ACC TTG ATC CGA CGT CTA CAG CTA TAT TCT CGA AAC CTG GAA CAT CTG GTA GAG
met asp thr leu ile arg arg leu gln leu tyr ser arg asn leu glu his leu val glu

2341/781 2371/791
GAA AGG ACA CAG CTG TAC AAG GCA GAG AGG GAC AGG GCT GAC AGA CTT AAC TTT ATG TTG
glu arg thr gln leu tyr lys ala glu arg asp arg ala asp arg leu asn phe met leu

2401/801 2431/811
CTT CCA AGG CTA GTG GTA AAG TCT CTG AAG GAG AAA GGC TTT GTG GAG CCG GAA CTA TAT
leu pro arg leu val val lys ser leu lys glu lys gly phe val glu pro glu leu tyr

2461/821 2491/831
GAG GAA GTT ACA ATC TAC TTC AGT GAC ATT GTA GGT TTC ACT ACT ATC TGC AAA TAC AGC
glu glu val thr ile tyr phe ser asp ile val gly phe thr thr ile cys lys tyr ser

2521/841 2551/851
ACC CCC ATG GAA GTG GTG GAC ATG CTT AAT GAC ATC TAT AAG AGT TTT GAC CAC ATT GTT
thr pro met glu val val asp met leu asn asp ile tyr lys ser phe asp his ile val

2581/861 2611/871
GAT CAT CAT GAT GTC TAC AAG GTG GAA ACC ATC GGT GAT GCG TAC ATG GTG GCT AGT GGT
asp his his asp val tyr lys val glu thr ile gly asp ala tyr met val ala ser gly

2641/881 2671/891
TTG CCT AAG AGA AAT GGC AAT CGG CAT GCA ATA GAC ATT GCC AAG ATG GCC TTG GAA ATC
leu pro lys arg asn gly asn arg his ala ile asp ile ala lys met ala leu glu ile

2701/901 2731/911
CTC AGC TTC ATG GGG ACC TTT GAG CTG GAG CAT CTT CCT GGC CTC CCA ATA TGG ATT CGC
leu ser phe met gly thr phe glu leu glu his leu pro gly leu pro ile trp ile arg

2761/921 2791/931
ATT GGA GTT CAC TCT GGT CCC TGT GCT GCT GGA GTT GTG GGA ATC AAG ATG CCT CGT TAT
ile gly val his ser gly pro cys ala ala gly val val gly ile lys met pro arg tyr

2821/941 2851/951
TGT CTA TTT GGA GAT ACG GTC AAC ACA GCC TCT AGG ATG GAA TCC ACT GGC CTC CCT TTG
cys leu phe gly asp thr val asn thr ala ser arg met glu ser thr gly leu pro leu

2881/961 2911/971
AGA ATT CAC GTG AGT GGC TCC ACC ATA GCC ATC CTG AAG AGA ACT GAG TGC CAG TTC CTT
arg ile his val ser gly ser thr ile ala ile leu lys arg thr glu cys gln phe leu

2941/981 2971/991
TAT GAA GTG AGA GGA GAA ACA TAC TTA AAG GGA AGA GGA AAT GAG ACT ACC TAC TGG CTG
tyr glu val arg gly glu thr tyr leu lys gly arg gly asn glu thr thr tyr trp leu

3001/1001 3031/1011
ACT GGG ATG AAG GAC CAG AAA TTC AAC CTG CCA ACC CCT CCT ACT GTG GAG AAT CAA CAG
thr gly met lys asp gln lys phe asn leu pro thr pro pro thr val glu asn gln gln

3061/1021 3091/1031
CGT TTG CAA GCA GAA TTT TCA GAC ATG ATT GCC AAC TCT TTA CAG AAA AGA CAG GCA GCA
arg leu gln ala glu phe ser asp met ile ala asn ser leu gln lys arg gln ala ala

3121/1041 3151/1051
GGG ATA AGA AGC CAA AAA CCC AGA CGG GTA GCC AGC TAT AAA AAA GGC ACT CTG GAA TAC
gly ile arg ser gln lys pro arg arg val ala ser tyr lys lys gly thr leu glu tyr

3181/1061 3211/1071
TTG CAG CTG AAT ACC ACA GAC AAG GAG AGC ACC TAT TTT TAA ACC TAA ATG AGG TAT AAG
leu gln leu asn thr thr asp lys glu ser thr tyr phe

3241 3271
GAC TCA CAC AAA TTA AAA TAC AGC TGC ACT GAG GCC AGG CAC CCT CAG GTG TCC TGA AAG

3301 3331
CTT ACT TTC CTG AGA CCT CAT GAG GCA GAA ATG TCT TAG GCT TGG CTG CCC TGT TTG GAC

3361 3391
CAT GGA CTT TCT TTG CAT GAA TCA GAT GTG TTC TCA GTG AAA TAA CTA CCT TCC ACT CTG

3421 3451
GAA CCT TAT TCC AGC AGT TGT TCC AGG GAG CTT CTA CCT GGA AAA GAA AAG AAT TTC ATT

3481 3511
TAT TTT TTG TTT GTT TAT TTT TAT CGT TTT TGT TTA CTG GCT TTC CTT CTG TAT TCA TAA

3541 3571
GAT TTT TTA AAT TGT CAT AAT TAT ATT TTA AAT ACC CAT CTT CAT TAA AGT ATA TTT AAC

3601 3631
TCA TAA TTT TTG CAG AAA ATA TGC TAT ATA TTA GGC AAG AAT AAA AGC TAA AGG TTT CCC

3661
AAA AAA AAA

CLAIMS

1. A vaccine composition comprising:

a) a protein comprising at least one epitope of human ST receptor protein or a nucleic acid molecule that encodes said protein; and

b) a pharmaceutically acceptable carrier or diluent.

2. The vaccine composition of claim 1 comprising said protein wherein said protein an epitope of the extracellular domain of the human ST receptor protein.

3. The vaccine composition of claim 2 comprising said protein wherein said protein comprises the extracellular domain of the human ST receptor protein.

4. The vaccine composition of claim 3 comprising said protein wherein the protein comprises the human ST receptor protein.

5. The vaccine composition of claim 4 comprising said protein wherein the protein consists of the human ST receptor protein.

6. The vaccine composition of claim 1 comprising a nucleic acid molecule that encodes said protein wherein said protein comprises an epitope of the extracellular domain of the human ST receptor protein.

7. The vaccine composition of claim 6 comprising a nucleic acid molecule that encodes said protein wherein said protein comprises the extracellular domain of the human ST receptor protein.

8. The vaccine composition of claim 7 comprising a nucleic acid molecule that encodes said protein wherein the protein comprises the human ST receptor protein.

9. The vaccine composition of claim 8 comprising a nucleic acid molecule that encodes said protein wherein the protein consists of the human ST receptor protein.

10. The vaccine composition of claim 1 comprising a nucleic acid molecule that encodes said protein wherein said nucleic acid molecule is a plasmid.

11. The vaccine composition of claim 1 comprising a nucleic acid molecule that encodes said protein wherein said nucleic acid molecule is within a viral vector or a bacterial cell.

12. The vaccine composition of claim 11 wherein said viral vector is a recombinant vaccinia virus.

13. A haptenized protein comprising at least one epitope of human ST receptor protein.

14. The haptenized protein of claim 13 wherein said protein comprises at least one epitope of the extracellular domain of the human ST receptor protein.

15. The haptenized protein of claim 14 wherein said protein comprises the extracellular domain of the human ST receptor protein.

16. The haptenized protein of claim 15 wherein the protein comprises the human ST receptor protein.

17. A vaccine composition that comprises a haptenized protein of claim 13 and a pharmaceutically acceptable carrier or diluent.

18. A vaccine composition comprising killed or inactivated cells or particles that comprise a protein comprising at least one epitope of human ST receptor protein and a pharmaceutically acceptable carrier or diluent.

19. The vaccine of claim 18 wherein said killed or inactivated cells or particles comprise a protein with an epitope of the extracellular domain of the human ST receptor protein.

20. The vaccine of claim 19 wherein said killed or inactivated cells or particles comprise a protein with the extracellular domain of the human ST receptor protein.

21. The vaccine of claim 20 wherein said killed or inactivated cells or particles comprise the human ST receptor protein.

22. The vaccine of claim 21 wherein said killed or inactivated cells or particles comprise killed or inactivated colorectal tumor cells.

23. The vaccine of claim 18 wherein said killed or inactivated cells or particles are haptenized killed or inactivated cells or particles.

24. A method of treating an individual who has metastasized colorectal cancer comprising the step of administering to such an individual a therapeutically effective amount of a vaccine of 1.

25. A method of treating an individual who has been identified as being susceptible to metastasized colorectal cancer comprising the step of administering to such an individual a prophylactically effective amount of a vaccine of 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07565

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/00; C12N 15/11; C07H 21/04; A61K 38/00
US CL : 530/350; 536/23.1, 23.5; 514/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.1, 23.5; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: ST receptor, guanylyl (or guanylin) cyclase C, colon or colonic or colorectal tumor, haptinized

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ALMENOFF et al. Induction of heat-stable enterotoxin receptor activity by a human Alu repeat. J. Biol. Chem. 17 June 1994, Vol. 269, No. 24, pages 16610-16617. See entire document, and references 15-16.	1-25
Y	CARRITHERS et al. Escherichia coli heat-stable toxin receptors in human colonic tumors. Gastroenterology. 1994, Vol. 107, pages 1653-1661. See entire document.	1-25
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07565

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MILLER et al. The induction of hapten-specific T cell tolerance by using hapten-modified lymphoid cells. J Immunol. November 1976, Vol. 117, No. 5, Part 1, pages 1519-1526. See entire document.	1-25